UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/734,936	12/12/2003	Wonchul Suh	CL1878USNA	2510

23906 7590 05/25/2006

E I DU PONT DE NEMOURS AND COMPANY
LEGAL PATENT RECORDS CENTER
BARLEY MILL PLAZA 25/1128
4417 LANCASTER PIKE
WILMINGTON, DE 19805

EXAMINER	
MCGILLEM, LAURA L	
ART UNIT	**PAPER NUMBER**
1636	

DATE MAILED: 05/25/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/734,936	SUH, WONCHUL	
	Examiner	Art Unit	
	Laura McGillem	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 March 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-11,13-24 and 26-30* is/are pending in the application.

4a) Of the above claim(s) *2,18 and 19* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 3-11, 13-17, 20-24, 26-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *12 December 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

It is noted that Applicants have amended claims 1, 3, 10-11, 15, 17 and 24 in the response filed 3/17/2006. Claims 12 and 25 have been canceled. Claims 1, 3-11, 13-17, 20-24 and 26-30 are under examination.

Claim Objections

Claim 28 is objected to because of the following informalities: it is dependent on claims 17-19 and claims 18-19 are drawn to non-elected inventions. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 3-11, 13-17, 20-24 and 26-30 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This is a new rejection.

Claims 1 and 17 are vague and indefinite because claims 1 and 17 have been amended to recite the phrase "RR3 is a third recombination element" in step b (ii) and then recite the phrase "having homology to the third recombination region" in step c)(ii) and since the claims only recite a third recombination element it is not clear to what third recombination *region* the claim intends.

Claim 3 is vague and indefinite because it recites the phrase "first or second expressible DNA fragment" and claim 3 is dependent from claim 1, which only recites the phrase " first expressible DNA fragment". Therefore, it is not clear what "second expressible DNA fragment" the Applicant intends.

Claims 9 and 23 recite the limitation "said regulatory region". There is insufficient antecedent basis for this limitation in the claim. Independent claims 8 and 22 recite "regulatory circuit" and not "regulatory region".

Claim 20 is vague and indefinite because it recites the phrase "said promoter" while independent claim 17 recites both foreign promoter and bacterial chromosome promoter and therefore it is not clear to which promoter the phrase "said promoter" refers.

Claim 17 is vague and indefinite because it has been amended to recite the phrase "recombinant proficient host cell of an *E.coli*" and it is not clear what cells would encompass being a host of an *E.coli*.

Claims 4-8, 10-16, 20-22, 24 and 26-30 are indefinite because they are dependent on indefinite claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the

invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 3, 7-11, 15-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Perkins et al (Application Publication No. 2002/0151058, of record) in view of Yu et al (of record) and further in view of Prideaux et al (U.S. Patent No. 6,472,183). This is a new rejection.

Applicant claims a method for the directed integration of an expressible DNA fragment lacking a selectable marker into an *E.coli* chromosome by providing a first and second recombination element such that both elements are integrated into a bacterial chromosome between a first and second chromosomal region, selecting and isolating the construct on the basis of the presence of the selectable marker and expressing a site specific recombinase wherein the selectable marker is excised from the chromosome.

Perkins et al teach recombination methods using intermediate expression vectors containing two sequence specific recombination regions (i.e. triple homologous recombination, see Figure 3). Perkins et al teach that the intermediate expression vectors can be linear. Perkins et al teach that a host cell can be a recombinant competent prokaryote such as *E.coli* (see paragraph 0010). Perkins et al teach that the method can involve homologous recombination with a host cell genome, which reads on a bacterial chromosome. Perkins et al teach that the first and second homologous recombination sequences can comprise selectable markers and a promoter. Perkins et al teach that the first and second elements can be produced using PCR amplification (see paragraph 0011, in particular). Perkins et al teach that the sequence specific

recombination regions can be at least about 15 nucleotides and an especially preferred size is from about 25 to about 60 nucleotides (see paragraphs 0023 and 0032, in particular) which reads on regions of about 10-50 bases. Perkins et al teach that target nucleic acids may include one or more genes and/or their associated regulatory regions (see paragraph 0033, in particular).

In Figure 3, Perkins et al teach non-contiguous linear fragments (301 and 302) that are not capable of recombining with a target sequence individually. Fragments 301 and 302 read on the claimed first and second recombination elements. Perkins et al teach that fragment 301 (first recombination element) can comprise a selectable marker (labeled 33) flanked by areas of homology (labeled 32 and 34), which reads on a structure of 5' RR1- Selectable Marker- RR2-3'. Perkins et al teach that fragment 302 (second recombination element) can comprise a promoter and a protein coding sequence (labeled 35 and 36) flanked by areas of homology (labeled 34 and 37), which reads on a structure of 5'-X(expressible element)- RR3-3'. Perkins et al illustrates that sequence 34 in fragment 301 (first recombination element) and sequence 34 in fragment 302 (second recombination element) are homologous to each other. Perkins et al illustrates that fragments 301 and 302 are recombined with each other at area 34 and with a target sequence (e.g. a bacterial genome) such that the structure is 5' 32-selectable marker -34-35-36-37-3', which is analogous to a construct having the general structure of 5' RR1-SM-RR2-X-RR3-3'. Perkins et al teach that selectable markers may be incorporated into the construct to facilitate detection of the introduced DNA in the host cell (see paragraph 0011, in particular).

Perkins et al do not teach that the construct comprises recombination sites (RS) flanking the selectable marker that are responsive to a site-specific recombinase, or the use of the λ Red recombination system, or a second recombinase reaction to eliminate the selectable marker.

Yu et al teach a recombination system for *E.coli* chromosomal engineering using a defective λ prophage in a temperature dependent system (i.e. λ Red system). Yu et al teach that this system depends on Exo, Beta and Gam gene functions expressed from the defective λ prophage. Yu et al teach that normal exonuclease activity readily degrades linear DNA in *E.coli* and complicates transformation of *E.coli* by linear constructs, but is overcome by the defective λ prophage genes. (see page 5978, left column 3rd paragraph). Yu et al teach this system is more efficient than other methods to allow *E.coli* to take up linear DNA and can be used with DNA homologies as short as 30-50 bp (see page 5978, right column, 2nd paragraph).

Yu et al do not teach that the construct comprises recombination sites flanking the selectable marker that are responsive to a site specific recombinase or a second recombinase reaction to eliminate the selectable marker.

It would have be obvious to one of ordinary skill in the art to modify the method of Perkins et al to include the use of the recombination system taught by Yu et al because Yu et al teach it is difficult to recombine linear DNA fragments into *E.coli* genomes and that this system is an improvement over the art at the time the invention was made. The motivation to use the λ Red recombination system is the expected benefit as exemplified by Yu et al of being able to recombine PCR-generated linear DNA

constructs comprising very short regions of homology into the *E.coli* genome in an efficient manner without additional steps that had been required using methods previously used in the art (see page 5982, right column, 1st and 2nd paragraphs, in particular). There is a reasonable expectation of success in using the λ Red recombination system because it has worked previously for Yu et al.

Prideaux et al teach a method of producing a toxin for use as a vaccine in a modified microorganism such as *E.coli*. Prideaux et al teach that in a method where recombinant DNA techniques are used, any method may be used to introduce foreign DNA into host cells (see column 2, lines 46-50 and column 3, lines 22-26, in particular). Prideaux et al teach that that it is useful to produce an RTX C gene for use as a vaccine that has been inactivated by introducing a targeting construct introduced by site-specific homologous recombination. Prideaux et al teach that it may be undesirable to have a functional antibiotic resistance gene incorporated into the modified microorganism. Prideaux et al discloses a targeting construct which includes genetic elements, such as repeat sequences or site specific recombination sites, which facilitate excision of the antibiotic resistance gene once the targeting construct has undergone homologous recombination with the host chromosome (see column 3, lines 41-62, column 6, lines 48-56 and column 18, lines 11-20, in particular). Prideaux et al teach that that the RTX C gene may also encode nucleotide sequences that facilitate transfer of the DNA to *E.coli* (see column 5, lines 30-42, for example). Prideaux et al teach that an introduced expressible gene (APX1 A) in their method is under the control of a regulatable

promoter/operator element consisting of the *E. coli* phage T5 promoter and two lac operator sequences.

It would have been obvious to one of ordinary skill in the art to modify the methods of Perkins et al to include a selectable marker flanked by recombination sequences so that the selectable marker could be excised from the host chromosome after homologous recombination because Perkins et al teach a method of introducing DNA sequences into a bacterial genome for production of genes of interest using selectable markers to screen for presence of the introduced vector and Prideaux et al teach that genes for antibiotic resistance used for selection can be undesirable after selection has taken place. The motivation to do so is the expected benefit as suggested by Prideaux et al of eliminating an undesirable functional antibiotic resistance gene associated with a desired gene of interest that already been confirmed to be integrated into the genome of the *E.coli*. There is reasonable expectation of success in using a selectable marker flanked by site-specific recombination sites to confirm chromosomal integration and then excise the selectable marker when it is no longer needed because this method has worked previously in the method taught by Prideaux et al. There is reasonable expectation of success in improving introduction of linear DNA into the *E.coli* genome by using the λ Red recombinase system and using an excisable selectable marker to confirm the presence of the DNA in the genome because these methods have worked previously in the cited techniques.

Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary

skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 1, 3-4, 7-8, 11, 13-17, 20-22 and 26-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Perkins et al (Application Publication No. 2002/0151058, of record) in view of Yu et al (of record) and further in view of Welch et al (Application Publication No. 2002/0187544) as evidenced by Guzman, et al (J. Bacteriol., 1995, 177(14): 4121-4130). This is a new rejection.

Applicant claims a method for the directed integration of an expressible DNA fragment lacking a selectable marker into an *E.coli* chromosome and a method for the integration of a foreign promoter in place of a bacterial chromosomal promoter in an *E.coli* cell. These methods comprise steps of providing a first and second recombination element such that both elements are integrated into a bacterial chromosome between a first and second chromosomal region, selecting and isolating the construct on the basis of the presence of the selectable marker and expressing a site specific recombinase wherein the selectable marker is excised from the chromosome.

As described above, Perkins et al teach recombination methods using intermediate expression vectors containing two sequence specific recombination regions (i.e. triple homologous recombination, see Figure 3). Perkins et al teach that the intermediate expression vectors can be linear. Perkins et al teach that a host cell can be a recombinant competent prokaryote such as *E.coli* (see paragraph 0010). Perkins et al teach that the method can involve homologous recombination with a host

cell genome. Perkins et al teach that the first and second homologous recombination sequences can comprise selectable markers and a promoter. Perkins et al teach that the first and second elements can be produced using PCR amplification (see paragraph 0011, in particular). Perkins et al teach that the sequence specific recombination regions can be at least about 15 nucleotides and an especially preferred size is from about 25 to about 60 nucleotides (see paragraphs 0023 and 0032, in particular). Perkins et al teach that target nucleic acids may include one or more genes and/or their associated regulatory regions (see paragraph 0033, in particular).

In Figure 3, Perkins et al teach non-contiguous linear fragments (301 and 302) that are not capable of recombining with a target sequence individually. Fragments 301 and 302 read on the claimed first and second recombination elements. Perkins et al teach that fragment 301 (first recombination element) can comprise a selectable marker (labeled 33) flanked by areas of homology (labeled 32 and 34), which reads on a structure of 5' RR1- Selectable Marker- RR2-3'. Perkins et al teach that fragment 302 (second recombination element) can comprise a promoter and a protein coding sequence (labeled 35 and 36) flanked by areas of homology (labeled 34 and 37), which reads on a structure of 5'-X(expressible element)- RR3-3'. Perkins et al illustrates that sequence 34 in fragment 301 (first recombination element) and sequence 34 in fragment 302 (second recombination element) are homologous to each other. Perkins et al illustrates that fragments 301 and 302 are recombined with each other at area 34 and with a target sequence (e.g. a bacterial genome) such that the structure is 5' 32-selectable marker -34-35-36-37-3', which is analogous to a construct having the general

structure of 5' RR1-SM-RR2-X-RR3-3'. Perkins et al teach that selectable markers may be incorporated into the construct to facilitate detection of the introduced DNA in the host cell (see paragraph 0011, in particular).

Perkins et al does not teach that a foreign promoter is integrated in the place of the native promoter. Perkins et al do not teach that the construct comprises recombination sites flanking the selectable marker that are responsive to a site specific recombinase, or the use of the λ Red recombination system, or a second recombinase reaction to eliminate the selectable marker.

Yu et al teach a recombination system for *E.coli* chromosomal engineering using a defective λ prophage in a temperature dependent system (i.e. λ Red system). Yu et al teach that this system depends on Exo, Beta and Gam gene functions expressed from the defective λ prophage. Yu et al teach that normal exonuclease activity readily degrades linear DNA in *E.coli* and complicates transformation of *E.coli* by linear constructs, but is overcome by the defective λ prophage genes (see page 5978, left column 3rd paragraph). Yu et al teach this system is more efficient than other methods to allow *E.coli* to take up linear DNA and can be used with DNA homologies as short as 30-50 bp (see page 5978, right column, 2nd paragraph).

Yu et al do not teach that the construct comprises recombination sites flanking the selectable marker that are responsive to a site specific recombinase or a second recombinase reaction to eliminate the selectable marker.

Welch et al teach a method of inserting genes into a bacterial chromosome to construct constitutive promoter mutants in *E.coli* (see paragraph 0029). Specifically,

Welch et al teach that the λ Red recombinase system and PCR generated recombinant fragments encoding an araBAD promoter (i.e. a foreign promoter) were used to replace a native promoter in order to control transcription of a dsdC gene (see paragraph 0063-0064, in particular). Welch et al cite Guzman et al as a reference regarding the araBAD promoter. Guzman et al teach that pBAD vectors (pBAD 18 and pBAD33) comprising a promoter and a gene comprising positive and negative regulators of the promoter (see page 4121, abstract and page 4122, Figure 1, for example) which reads on a foreign promoter that comprises positive and negative regulatory sites.

Welch et al teach an embodiment of their method that uses the λ Red recombinase system and comprising PCR generation of 36-50 nucleotide extensions that are homologous to a chromosomal region adjacent to the gene of interest. Welch et al teach in corporation of an antibiotic resistance gene flanked by FLP resistance target sites. Welch et al further teach that the PCR generated nucleotide sequence was introduced into a cell carrying a cell with the Red expression plasmid, and selected via the activity of the antibiotic resistance gene. Welch et al teach that the resistance plasmid was excised by introduction of a helper FLP plasmid that acts on the FLP recognition target sites (see paragraph 0049, in particular) which reads on the method wherein the site specific recombinase is FLP recognizing the *frt* recombination site.

It would have be obvious to one of ordinary skill in the art to modify the method of Perkins et al to include the use of the recombination system taught by Yu et al because Yu et al teach it is difficult to recombine linear DNA fragments into *E.coli* genomes and that this system is an improvement over the art at the time the invention was made. It

would also have been obvious to one of ordinary skill in the art to modify the methods of Perkins et al by incorporating the method of Welch et al which comprises the λ Red recombinase system to replace a native promoter with a foreign constitutive promoter using an excisable selectable marker system because Perkins et al teaches methods of introducing promoters and genes of interest into a bacterial chromosome and the λ Red recombinase system is a more efficient method to incorporate linear DNA into a bacterial chromosome (as exemplified by Yu et al). Welch teaches that expression of the gene of interest can be examined independently of the native promoter, which is dependent on D-serine. The motivation to do so is the expected benefit of being able to accurately examine a variety of phenotypes created by various deletions and mutations independently from D-serine induced expression (i.e. any induced signaling necessary for activation of the native promoter) (see paragraph 0065, in particular). The motivation to use the λ Red recombination system is the expected benefit as exemplified by Yu et al of being able to recombine linear DNA constructs comprising very short regions of homology into the *E.coli* genome in an efficient manner. There is reasonable expectation of success in combining two linear elements as in Perkins in methods of homologous recombination to incorporate foreign promoters and genes into a bacterial chromosome using the λ Red recombination system since these methods have worked previously in the cited techniques.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura McGillem whose telephone number is (571) 272-8783. The examiner can normally be reached on M-F 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Irem Yucel can be reached on (571) 272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Laura McGillem, PhD
5/16/2006

DANIEL M. SULLIVAN
PATENT EXAMINER